

# Human spleen DNA

ag N —  $^{32}P$  2633 (into the anchor primer)  
 follow P. 53 except use more  $^{32}P$  ATP ~26% primers have ATP is 100% efficiency in labeling

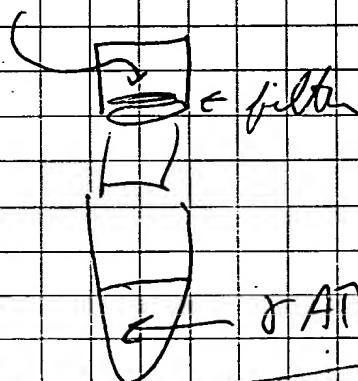
159 $\mu M$ $^{32}P$ ATP 6000 Ci/mmol 10 $\mu Ci/\mu l$ 10-21-94 (1.67 $\mu M$ ATP) 5x Kinase buffer PNK 50 $\mu l$	(1) 1' $\mu l$ ✓ 25' $\mu l$ ✓ 675 ✓ 0.25 $\mu l$ ✓ 33.75	(159 $\mu M$ primer) (2) 1' $\mu l$ ✓ 25' $\mu l$ ✓ 675 ✓ 0.25 $\mu l$ ✓ 33.75	Any down 1126 ladder 10 $\mu l$ H <sub>2</sub> O 1 $\mu l$ 34P DP 15' 37°C 1 $\mu l$ EDTA
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37°C 30 min → 5' 55°C → add

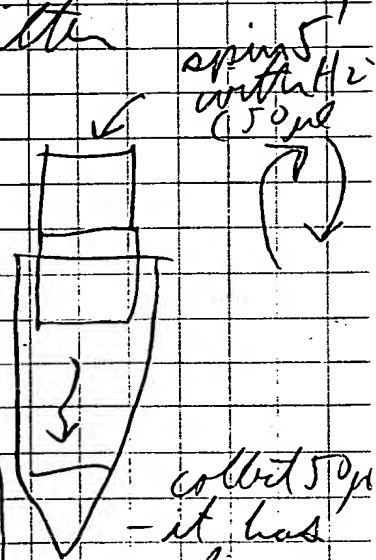
spin col same as P154, 7, and 145, 3

dilute  $^{32}P$  2633 with 100  $\mu l$  H<sub>2</sub>O (Vp = 133 now)

spin in microfuge in "micron 3" (amicon # 42402) - after all went in, put add 200  $\mu l$  more H<sub>2</sub>O and spin again  
 remove volume that did not enter filter



invert filter



10-24-94

Had a problem: filter kept packing back on micron 3. Maybe g force was too high on Beckman microfuge "E" model will skip separation of free ATP.

$^{32}P$  2633 is diluted only 33.75 fold for CF = 4.71  $\mu M$

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Read & Understood by me, [Signature]	Date 10/24/94	Invented by [Signature]	Date 10-19-94 10/24/94
		Recorded by	

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32P 2.633 4.71  $\mu$ M①  
✓ 4.7  $\mu$ l②  
4.7  $\mu$ l0.2  $\mu$ l2.628 cold 199  
dilute to 10  $\mu$ M✓ 2.2  $\mu$ l →0.2  $\mu$ M80 ng/ $\mu$ l Human  
spleen DNA✓ 1.1  $\mu$ l →

(80 ng/10

4 dNTPs 10 mM each

✓ 2.2  $\mu$ l →200  $\mu$ M

pol mix

Vent 2  $\mu$ l/ $\mu$ l0.5  $\mu$ lTf1 1  $\mu$ l/ $\mu$ l15  $\mu$ lVp = 15.5  $\mu$ l →✓ 1.36  $\mu$ l 4.08

Total

(1.32 unit  
0.08  $\mu$ l Vent  
in ①. 3  
more in ② =  
0.28  $\mu$ l Vent5X buffer  
Cheng

✓ 22 →

Mg (OAc)<sub>2</sub>  
12 mM

✓ 11 →

Cp = 1.2

H<sub>2</sub>O

32.2/15.5

65.44

62.7

✓ 75.34

72.6

Vp = 110  $\mu$ l110  $\mu$ lremove 10  $\mu$ l to 2  $\mu$ l 0.2 M EDTA at 0 cycles.  
remove 10  $\mu$ l at 5, 10, 15, 20, 25, 30, 36

program

13"

15", 94°C → 20 min, 68°C

140

10", 72°C

141

1", 94°C

started at 8:16 AM  
20.5 min/cycleneed 12 hr, 20 min to  
complete so expect to end  
at 8:40 - 9:00 PM

142 = 141, 139, 140, 4

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Date

10/24/94

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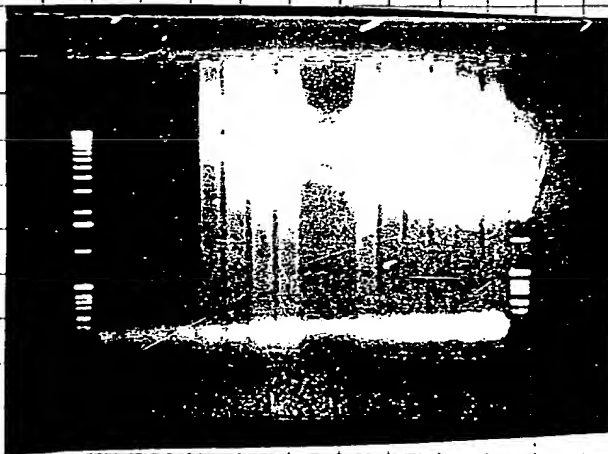
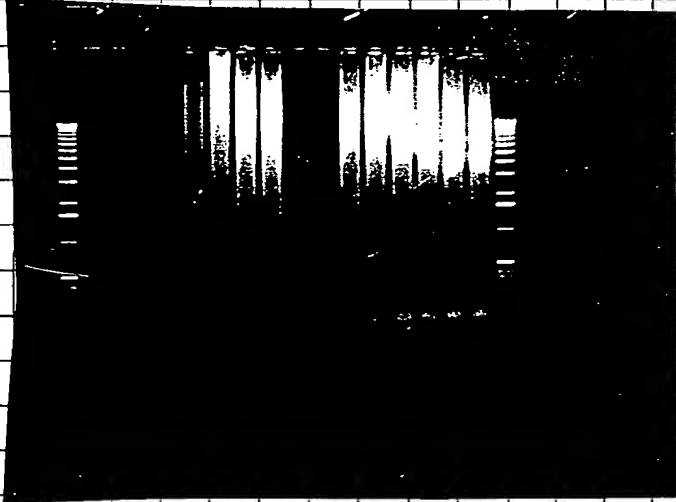
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10-24-94

Page No. — 8 1/2 square same as P. 56

1.33 4

05101120217036 05101120217036



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Read & Understood by me,

*Erica Bokup*

Date

10/24/94

Invented by

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Date